We claim:

1. A method of diagnosing the presence or severity of liver fibrosis in an individual, comprising the steps of:

(a) detecting α2-macroglobulin in a sample from said individual;

(b) detecting hyaluronic acid (HA) in a sample from said individual;

(c) detecting tissue inhibitor of metalloproteinases-1 (TIMP-1) in a sample from said individual; and

(d) diagnosing the presence or severity of liver fibrosis in said individual based on the presence or level of α2-MG, HA and TIMP-1.

2. The method of claim 1, comprising detecting at most three markers of fibrosis.

3. The method of claim 1, further comprising detecting in a sample from said individual at least one marker selected from the group consisting of: PIIINP, laminin, tenascin, collagen type IV, collagen type VI, YKL-40, MMP-3, MMP-2, MMP-9/TIMP-1 complex, sFas ligand, TGF-β1, IL-10, apoA1, apoA2, and apoB.

4. The method of claim 3, wherein said marker is YKL-40.

5. The method of claim 1, further comprising detecting in a sample from said individual two or more markers selected from the group consisting of PIIINP, laminin, tenascin, collagen type IV, collagen type VI, YKL-40, MMP-3, MMP-2, MMP-9/TIMP-1 complex, sFas ligand, TGF-β1, IL-10, apoA2, apoA2 and apoB.

6. The method of claim 1, wherein said individual has viral hepatitis.

7. The method of claim 7, wherein said individual is infected with hepatitis C virus.

8. The method of claim 7, wherein said individual is infected with hepatitis B virus.

9. The method of claim 1, wherein said individual has autoimmune liver disease.

10. The method of claim 1, wherein said individual has alcoholic liver disease.

11. The method of claim 1, wherein said individual has a fatty liver disease.

12. The method of claim 1, wherein said individual has drug-induced liver disease.

13. The method of claim 1, wherein step (a) comprises determining the level of α2-MG protein in said sample.

14. The method of claim 13, wherein the level of α2-MG protein is determined using one or more α2-MG-specific binding agents.

15. The method of claim 14, wherein the level of α2-MG protein is determined using one or more anti-α2-MG antibodies.

16. The method of claim 1, wherein step (a) comprises determining a level of α2-MG activity.

17. The method of claim 1, wherein step (b) comprises determining the level of HA in said sample.

18. The method of claim 17, wherein the level of HA is determined using one or more HA-specific binding agents.

19. The method of claim 18, wherein the level of HA is determined using one or more HA-binding proteins.

20. The method of claim 18, wherein the level of HA is determined using one or more anti-HA antibodies.

21. The method of claim 1, wherein step (c) comprises determining the level of TIMP-1 protein in said sample.

22. The method of claim 21, wherein the level of TIMP-1 protein is determined using one or more TIMP-1-specific binding agents.

23. The method of claim 22, wherein the level of TIMP-1 protein is determined using one or more anti-TIMP-1 antibodies.

24. The method of claim 1, wherein step (c) comprises determining a level of TIMP-1 activity.

25. The method of claim 1,

wherein step (a) comprises determining the level of α2-MG protein,

wherein step (b) comprises determining the level of HA, and

wherein step (c) comprises determining the level of TIMP-1 protein.

26. The method of claim 25, wherein the level of α2-MG protein, HA and TIMP-1 protein each is determined using an enzyme-linked assay.

27. The method of claim 1, wherein a single sample is obtained from said individual.

28. The method of claim 27, wherein said sample is selected from the group consisting of blood, serum, plasma, urine, saliva and liver tissue.

29. The method of claim 28, wherein said sample is a serum sample.

30. The method of claim 1, comprising differentiating no or mild liver fibrosis from moderate to severe liver fibrosis.

31. A method of differentiating no or mild liver fibrosis from moderate to severe liver fibrosis in an individual, comprising the steps of:

(a1) contacting an appropriate dilution of a sample from said individual with anti-α2-MG antibody under conditions suitable to form a first complex of α2-MG and anti-α2-MG antibody;

(b) washing said first complex to remove unbound molecules;

(c) determining the amount of α2-MG-containing first complex;

(d) contacting an appropriate dilution of a sample from said individual with a HA-binding protein (HABP) under conditions suitable to form a second complex of HA and HABP;

(e) washing said second complex to remove unbound molecules;

(f) determining the amount of HA-containing second complex;

(g) contacting an appropriate dilution of a sample from said individual with anti-TIMP-1 antibody under conditions suitable to form a third complex of TIMP-1 and anti-TIMP-1 antibody;

(h) washing said third complex to remove unbound molecules;

(i) determining the amount of TIMP-1-containing third complex; and

(j) differentiating no/mild liver fibrosis from moderate/severe liver fibrosis in said individual based on the amounts of α2-MG, HA and TIMP-1-containing complexes.

32. A method of monitoring the efficacy of anti-fibrotic therapy in a patient, comprising the steps of:

(a) detecting α2-macroglobulin in a sample from a patient administered an anti-fibrotic therapy;

(b) detecting hyaluronic acid (HA) in a sample from said patient;

(c) detecting tissue inhibitor of metalloproteinases-1 (TIMP-1) in a sample from said patient; and

(d) determining the presence or severity of liver fibrosis in said patient based on the presence or level of α2-MG, HA and TIMP-1, thereby monitoring the efficacy of anti-fibrotic therapy.

33. The method of claim 32, further comprising comparing the presence or severity of liver fibrosis determined in step (d) to the presence or severity of liver fibrosis in said patient at an earlier time.

34. The method of claim 32, comprising detecting at most three markers of fibrosis.

35. The method of claim 32, further comprising detecting in a sample from said patient at least one marker selected from the group consisting of: PIIINP, laminin, tenascin, collagen type IV, collagen type VI, YKL-40, MMP-3, MMP-2, MMP-9/TIMP-1 complex, sFas ligand, TGF-β1, IL-10, apoA1, apoA2, and apoB.

36. The method of claim 32, wherein step (a) comprises determining the level of α2-MG protein in said sample.

37. The method of claim 36, wherein the level of α2-MG protein is determined using one or more anti-α2-MG antibodies.

38. The method of claim 32, wherein step (b) comprises determining the level of HA in said sample.

39. The method of claim 38, wherein the level of HA is determined using one or more HA-binding proteins.

40. The method of claim 32, wherein step (c) comprises determining the level of TIMP-1 protein in said sample.

41. The method of claim 40, wherein the level of TIMP-1 protein is determined using one or more anti-TIMP-1 antibodies.

42. A method of differentiating no/mild liver fibrosis from moderate/severe liver fibrosis in an individual, comprising the steps of:

(a) determining an α2-MG level in a sample from said individual;

(b) determining a HA level in a sample from said individual;

(c) determining a TIMP-1 level in a sample from said individual; and

(d) diagnosing said individual as having no/mild liver fibrosis when said α2-MG level is below an α2-MG cut-off value X1, said HA level is below a HA cut-off value Y1 or said TIMP-1 level is below a TIMP-1 cut-off value Z1,

diagnosing said individual as having moderate/severe liver fibrosis when said α2-MG level is above an α2-MG cut-off value X2, said HA level is above a HA cut-off value Y2 and said TIMP-1 level is above a TIMP-1 cut-off value Z2,

and diagnosing remaining individuals as having an indeterminate status.

43. The method of claim 42, wherein said individual has a disorder selected from the group consisting of viral hepatitis, autoimmune liver disease, alcoholic liver disease, fatty liver disease and drug-induced liver disease.

44. The method of claim 43, wherein said individual is infected with hepatitis C virus.

45. The method of claim 42, wherein said samples are independently selected from the group consisting of blood, serum, plasma, urine, saliva and liver tissue.

46. The method of claim 45, wherein said α2-MG, level, HA level and TIMP-1 level each is determined in a serum sample.

47. The method of claim 46,

wherein X1 is a value between 1.8 and 2.2 mg/ml;

wherein Y1 is a value between 31 and 39 ng/ml;

wherein Z1 is a value between 900 and 1100 ng/ml;

wherein X2 is a value between 1.8 and 2.2 mg/ml;

wherein Y2 is a value between 54 and 66 ng/ml; and

wherein Z2 is a value between 1415 and 1735 ng/ml.

48. The method of claim 47,
wherein X1 = 2.0 mg/ml;
wherein Y1 = 35 ng/ml;
wherein Z1 = 1000 ng/ml;
wherein X2 = 2.0 mg/ml;
wherein Y2 = 60 ng/ml; and
wherein Z2 = 1575 ng/ml.

49. The method of claim 47,
wherein X1 = 2.0 mg/ml;
wherein Y1 = 37 ng/ml;
wherein Z1 = 1100 ng/ml;
wherein X2 = 2.0 mg/ml;
wherein Y2 = 60 ng/ml; and
wherein Z2 = 1575 ng/ml.

50. The method of claim 42, wherein, in a population having up to 30% liver fibrosis prevalence, at least 65% of individuals in said population are diagnosed as having no/mild fibrosis or moderate/severe fibrosis with an accuracy of at least 80%.

51. The method of claim 42, wherein, in a population having up to 30% liver fibrosis prevalence, at least 65% of individuals in said population are diagnosed

as having no/mild fibrosis or moderate/severe fibrosis with an accuracy of at least 90%.

52. The method of claim 42, wherein, in a population having up to 30% liver fibrosis prevalence, at least 65% of individuals in said population diagnosed as having no/mild fibrosis or moderate/severe fibrosis with a positive predictive value of at least 90% and a negative predictive value of at least 90%.

53. The method of claim 42, wherein, in a population having up to 10% liver fibrosis prevalence, at least 70% of individuals in said population are diagnosed as having no/mild fibrosis or moderate/severe fibrosis with an accuracy of at least 90%.

54. A method of diagnosing the presence or severity of liver fibrosis in an individual, comprising the steps of:

(a) comparing a level of a first fibrotic marker X in said individual to a cut-off value X1 to determine whether said individual is positive for said first fibrotic marker X;

(b) comparing a level of a second fibrotic marker Y in said individual to a cut-off value Y1 to determine whether said individual is positive for said second fibrotic marker Y; and

(c) diagnosing the presence or severity of liver fibrosis in said individual based on positivity or negativity for X and Y,

wherein, in a population with up to 40% fibrosis prevalence, at least 65% of individuals in said

population are diagnosed with an accuracy of at least 90%.

55. The method of claim 54, further comprising

(d) comparing a level of a third fibrotic marker Z in said individual to a cut-off value Z1 to determine whether said individual is positive for said third fibrotic marker Z; and

(e) diagnosing the presence or severity of liver fibrosis in said individual based on positivity or negativity for X, Y and Z.

56. The method of claim 55, wherein said first fibrotic marker is α2-MG, said second fibrotic marker is HA, and said third fibrotic marker is TIMP-1.

57. The method of claim 55, wherein the levels of at least three fibrotic markers are compared.

58. The method of claim 55, wherein the levels of three fibrotic markers are compared.

59. The method of claim 55, wherein the levels of at least four fibrotic markers are compared.

60. The method of claim 55, wherein the levels of at least five fibrotic markers are compared.

61. The method of claim 54, wherein said diagnosis differentiates no or mild liver fibrosis from moderate to severe liver fibrosis.

62. The method of claim 54 or claim 61, wherein, in a population with up to 30% fibrosis prevalence, at least 65% of individuals in said population are diagnosed with an accuracy of at least 93%.

63. The method of claim 54 or claim 61, wherein, in a population with up to 20% fibrosis prevalence, at least 70% of individuals in said population are diagnosed with an accuracy of at least 94%.

64. The method of claim 54 or claim 61, wherein, in a population with up to 10% fibrosis prevalence, at least 70% of individuals in said population are diagnosed with an accuracy of at least 96%.

65. A method of diagnosing the presence or severity of liver fibrosis in an individual, comprising the steps of:

(a) comparing a level of a first fibrotic marker X in said individual to a cut-off value X1 to determine whether said individual is positive for said first fibrotic marker X;

(b) comparing a level of a second fibrotic marker Y in said individual to a cut-off value Y1 to determine whether said individual is positive for said second fibrotic marker Y; and

(c) diagnosing the presence or severity of liver fibrosis in said individual based on positivity or negativity for X and Y,

wherein said cut-off values X1 and Y1 are optimized individually to give a desired performance characteristic.

66. The method of claim 65, further comprising

(d) comparing a level of a third fibrotic marker Z in said individual to a cut-off value Z1 to determine whether said individual is positive for said third fibrotic marker Z; and

(e) diagnosing the presence or severity of liver fibrosis in said individual based on positivity or negativity for X, Y and Z,

wherein said cut-off values X1, Y1 and Z1 are optimized individually to give a desired performance characteristic.

67. The method of claim 66, wherein said first fibrotic marker is α2-MG, said second fibrotic marker is HA, and said third fibrotic marker is TIMP-1.

68. The method of claim 65, wherein said cut-off values are optimized using design of experiments (DOE) analysis.

69. The method of claim 66, wherein the levels of at least three fibrotic markers are compared.

70. The method of claim 66, wherein the levels of three fibrotic markers are compared.

71. The method of claim 65, wherein said diagnosis differentiates no or mild liver fibrosis from moderate to severe liver fibrosis.

72. A method of diagnosing the presence or severity of liver fibrosis in an individual, comprising the steps of:

(a) comparing a level of a first fibrotic marker X in said individual to two cut-off values X1 and X2 to determine whether said individual is positive for said first fibrotic marker X;

(b) comparing a level of a second fibrotic marker Y in said individual to two cut-off values Y1 and Y2 to determine whether said individual is positive for said second fibrotic marker Y; and

(c) diagnosing the presence or severity of liver fibrosis in said individual based on positivity or negativity for X and Y,

wherein said cut-off values X1, Y1, X2 and Y2 are optimized individually to give a desired performance characteristic.

73. The method of claim 72, further comprising

(d) comparing a level of a third fibrotic marker Z in said individual to two cut-off values Z1 and Z1 to determine whether said individual is positive for said third fibrotic marker Z; and

(e) diagnosing the presence or severity of liver fibrosis in said individual based on positivity or negativity for X, Y and Z,

wherein said cut-off values X1, Y1, Z1, X2, Y2 and Z2 are optimized individually to give a desired performance characteristic.

74. The method of claim 73, wherein said cut-off values are optimized using design of experiments (DOE) analysis.